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Morphological separation of the Central European *Trochosa* females (Araneae, Lycosidae)

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Abstract: Adult females of the five Central European wolf spiders *Trochosa hispanica* Simon, 1870, *T. robusta* (Simon, 1876), *T. ruricola* (De Geer, 1778), *T. spinipalpis* (F. O. P.-Cambridge, 1895), and *T. terricola* Thorell, 1856 were morphologically analysed. We defined sets of continuous and binary (presence/absence) variables. Continuous data of various epigynal and carapace dimensions were subjected to Principal Components Analysis (PCA). Using the PC loadings each individual was plotted along the PC axis in order to find gaps/overlaps between the species. The binary data sets were subjected to Hierarchical Cluster Analysis (HCA) in order to find characters that clearly separate the five *Trochosa* species. Using PCA only individuals of *T. robusta* and *T. ruricola* and of *T. robusta* and *T. hispanica* could be separated from each other. Using HCA all five species could clearly be separated by epigynal and vulval characteristics.

key words: morphometry, spiders, taxonomy

Five species of the wolf spider genus *Trochosa* C. L. Koch, 1847 occur in Central Europe: *T. hispanica* Simon, 1870, *T. robusta* (Simon, 1876), *T. ruricola* (De Geer, 1778), *T. spinipalpis* (F. O. P.-Cambridge, 1895) and *T. terricola* Thorell, 1856. Having examined four *Trochosa* species (not considering *T. hispanica*) ENGELHARDT (1964) found that females are very similar in size and epigynal structures and have thus to be considered sibling species. While male *Trochosa* species can readily be separated, the determination of the females, unfortunately, is difficult to near impossible (HEIMER & NENTWIG 1991). Several attempts to distinguish preserved *Trochosa* material have been undertaken (e.g. DAHL 1908, CHRYSANTHUS 1955, BUCHAR 1959, ENGELHARDT 1964, MILASOWSZKY et al. 1998). LOCKET & MILLIDGE (1951) separated *T. robusta*, *T. ruricola*, *T. spinipalpis* and *T. terricola* females on the basis of colouration and the ratio of certain measurements of the epigyne and carapace, for example the ratio between the width of the sternum measured between coxae II and the width of the triangular septum. BUCHAR (1959, see Fig. 1) separated the species according to the position and form of the "Seitenhöcker" and the form of the "mittlere Lamelle". ENGELHARDT (1964) concluded that body colouration, especially in females, is the only reliable character which distinguishes the species. However, in recent determination keys genitalic characters are employed to separate the females, at least to some extent (e.g. TANAKA 1988, ROBERTS 1995). ROBERTS (1985) admitted that the "overall impression" of the epigynal structures yields more information than comparison of single parts. However, MILASOWSZKY et al. (1998) demonstrated that a clear separation of *T. robusta* and *T. ruricola* is possible by morphological/morphometrical analysis of somatic and genitalic characters.

The present study expands upon the findings of MILASOWSZKY et al. (1998) by considering additional characters and taxa of *Trochosa*. The aim was to find reliable morphological characters, both of the epigynes and vulvae that clearly separate the females of the five Central European *Trochosa* species.

Material and Methods

In the present study, 207 *Trochosa* specimens and their epigynes were examined, of which 48 vulvae were carefully prepared (*T. hispanica* n = 28/4 vulvae, *T. robusta* n = 43/6, *T. ruricola* n = 48/21, *T. spinipalpis* n = 34/7 and *T. terricola* n = 54/10). The material used was kindly provided by J. Gruber (Natural History Museum of Vienna), A. Hänggi (Natural History Museum Basel), P. Jäger, (Forschungsinstitut und Naturmuseum Senckenberg),

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C. Komposch (private collection), N. Milasowszky (private collection), and P. Schwendinger (Ville de Genève, Muséum d'histoire naturelle). Reliably identified species came either from single species populations or from studies where species have already been clearly separated. The status of tentatively assigned and misidentified specimens was corrected after our examination.

Material examined

Forschungsinstitut und Naturmuseum Senckenberg (with SMF coll. No.): *T. hispanica*: F., Korsika, 9039/1, 1♀; 9033/1, 1♀; 8979/1, 1♀; 9077/1, 1♀; 8989/2, 1♀; I., Sardinien, 9006/2, 2♀♀; 9011/2, 1♀; 9083/1, 1♀; 9005/1, 1♀; *T. spinipalpis*: D., Kaiserstuhl, 28628, 3♀♀; D., Oberbayern, 33059, 7♀♀; D., Rheinland-Pfalz, Draisberghof, P. Jäger.

Komposch, C.: *T. spinipalpis*: A., Kärnten, near Hüttenberg, Hörfeld-Moor, 13.VI.1996, 5♀♀.

Milasowszky, N.: *T. ruricola*: A., Burgenland, Seewinkel, 42♀♀; *T. robusta*: A., Burgenland, Seewinkel, 17♀♀; *T. terricola*: A., Vienna, Lobau, 18♀♀.

Natural History Museum Basel (with Coll. No.): *T. hispanica*: 2373p, CH, Tessin, 29.VI.1988, 1♀; 2373l, CH, Tessin, 20.VII.-04.VIII.1989, 2♀♀; 2373c, CH, Tessin, V-VI.1890, Coll. E. Schenkel, 6♀♀; 2373b, CH, Tessin, 1918, Coll. E. Schenkel, 1♀; 2373i, CH, Tessin, 30.IV.-14.V.1990, 2♀♀; *T. robusta*: 250a, CH, Umgebung Basel, 1♀; 250g, CH, Wallis, 28.V.-04.VI.1991, 1♀; 250d, CH, Umgebung Basel, 1892, Coll. E. Schenkel, 8♀♀; 250n, CH, Basel, 15.IV.-29.IV.2002, 2♀♀; *T. spinipalpis*: 2152m, CH, Aargau, 1972, 1♀; 2152l, CH, Jura, 27.VIII.1988, 1♀; 2152g, SK, Hohe Tatra, 31.VII.1982, 1♀; 2152j, CH, Waadt, 29.IV-06.V.1994, 2♀♀; 2152i, F, Elsass, 18.IV.-26.IV.1994, 1♀; 2152d, CH, Nidwalden, 25.VII.1942, 2♀♀; 2152c, D, Mecklenburg, 2♀♀; 2152k, CH, Obwalden, 31.V.92, 1♀; 2152f, A, Tirol, 1♀; 2152e, CH, Jura, IX.1943, 1♀.

Natural History Museum of Vienna (with Acqu.-No.): *T. hispanica*: Gr., Makedonia, Chalkidiki-E, 2000.XII.30., 2♀♀; Gr., Makedonia, Krekini-Gebirge, 2000.XII.29., 1♀; I., Südtirol. 1990.XX.14., 2♀♀; *T. robusta*: A., Burgenland, Pandorfer Platte, 1990.XXX., 3♀♀; A., Steiermark, Kalsdorf, 1986.XXII., 2♀♀; *T. ruricola*: A., Vienna, 1981.XX., 3♀♀; A., Nordtirol, Innsbruck-Umgebung, Rinn, 900m, 1987.VI.1, 2♀♀; *T. spinipalpis*: A., Niederösterreich, Krems, 2♀♀; *T. terricola*: A., Burgenland, Pandorfer Platte, 1990.XXX., 5♀♀; A., Nordtirol, Innsbruck-Umgebung, Rinn, 900m, 1987.VI.1, 14♀♀; A., Wien/Niederösterreich, Wienerwald, 5♀♀ (priv. coll. Gruber).

Ville de Genève, Muséum d'histoire naturelle: Material: Araneae: Lycosidae, F.36, S.f.2: *T. hispanica*: CH, Ticino, Melera, Vorwand, 11.IX-24.IX.1988, det. A. Hänggi, 1♀; *T. spinipalpis*, Russia, Bashkiria, Ufa, park, VII. 1979, leg. Bliss, ex coll. S. Heimer, 2♀♀; D, Dresden, ex. coll. S. Heimer, 2♀♀; CH, Altenburg, 2♀♀.

Morphological/morphometrical analysis

The entire material was preserved in ~70% alcohol. Remaining tissues from the prepared vulvae were removed with pins and by boiling in 4% KOH.

A total of 28 characters were examined from the material including ten continuous (interval-scaled), two categorical (ordinal-scaled) and 16 binary (presence/absence) characters (Tab. 1 & 2). The continuous characters (Fig. 1, A-H, Tab. 1) comprise two variables of the carapace and eight of the epigyne (n = 207). The two categorical characters are the inner and outer row of the cheliceral margin.

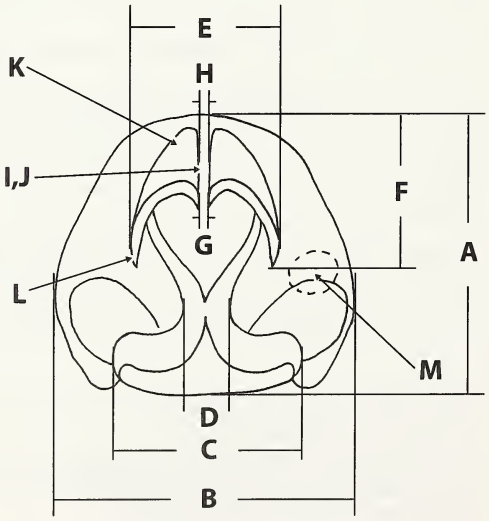

Fig. 1: Schematic drawing of a *Trochosa* epigyne showing the continuous (A-H) and binary (presence/absence) (I-M) variables used in this study. Distances show length of continuous variables, arrows point to binary characters. **A** = height of epigynal plate; **B** = width of epigynal plate; **C** = maximum width of triangular septum; **D** = outer distance between septal margins before expanding posteriorly into triangular septum; **E** = maximum distance between arches of anterior transverse pockets (= posterior part of helmet-shaped broadenings of vulva); **F** = distance between distal part of transverse pockets and distal transverse edge of epigynal plate; **G** = medial distance between transverse pockets; **H** = distance between inner edges of distal part of transverse pockets; **I** = inner margin of helmet-shaped broadenings of vulva; **J** = course of inner margins of helmet-shaped broadenings of vulva; **K** = helmet-shaped broadenings of vulva; **L** = shape of dark markings anterior to transverse pockets; **M** = appendices of basal part of copulatory ducts.

Among the binary characters eleven were obtained from the epigyne (Fig. 1, Tab. 2) (n = 205) and five from the vulva (Fig. 2, Tab. 2) (n = 48).

Statistical analysis

The continuous characters (Fig. 1 & Tab. 1, A-H) were subjected to Principal Components Analysis (PCA) using the correlation matrix and varimax rotation solution. Only principal components that accounted for variances greater than one (Kaiser criterion) were used to represent the data.

The binary characters of the epigyne and vulva (Fig. 1 & 2, Tab. 2) were separately subjected to Hierarchical Cluster Analysis (HCA) using the average linkage between groups (UPGMA) as a cluster method. Two different measurements were used: (i) the squared Euclidian distance for the set of continuous variables and (ii) the Lance and Williams index for the binary data set.

All statistical analyses were preformed using SPSS for Windows, Version 11.5 (NORUISIS 1990).

Results

Principal Component Analysis

Principal Component Analysis (PCA) of the 10 continuous characters (Fig. 1, Tab. 1) yielded two factors, PC1 and PC2. The first principal component, PC1, accounted for 55.7% of the total variation. Characters highly correlated with this axis are: (1) cl, the length of the carapace; (2) cw, the width of the carapace; (3) A, height of epigynal plate; (4) B, width of epigynal plate; (5) C, maximum width of triangular septum; and (6) E, maximum distance between the arches of the anterior transverse pockets.

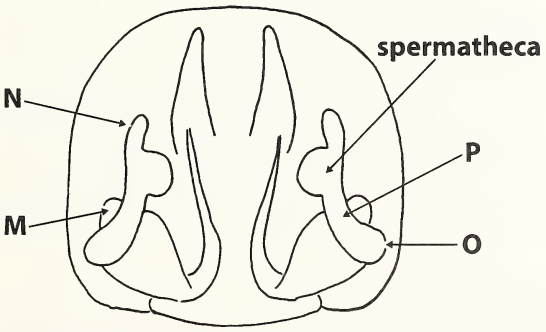

Fig. 2: Schematic drawing of a vulva showing the binary (presence/absence) (M-P) variables used in this study. **M** = appendices of basal part of copulatory ducts; **N** = appendix of spermatheca; **O** = lateral enlargement of copulatory duct; **P** = copulatory duct.

Tab. 1: Continuous characters measured on the carapace and epigynes of *T. hispanica* (n = 28), *T. robusta* (n = 43), *T. ruricola* (n = 48), *T. spinipalpis* (n = 34) and *T. terricola* (n = 54).

Continuous characters	
cl	length of carapace
cw	width of carapace
A	height of epigynal plate
B	width of epigynal plate
C	maximum width of triangular septum
D	outer distance between the septal margins before expanding posteriad into the triangular septum
E	maximum distance between the arches of the anterior transverse pockets (= posterior part of the helmet-shaped broadenings of the vulva)
F	distance between the distal part of the transverse pockets and the distal transverse edge of the epigynal plate
G	medial distance between the transverse pockets
H	distance between the inner edges of the distal part of the transverse pockets

The second principal component, PC2, accounted for 14.7% of the variation. Characters highly correlated with this axis are: (1) F, distance between the distal part of the transverse pockets and the distal transverse edge of the epigynal plate; (2) H, distance between the inner edges of the distal part of the transverse pockets; and (3) G, medial distance between the transverse pockets. Both principal components together accounted for 70.3% of the total variation in the morphometrical data set (Fig. 1, Tab. 1). A clear separation of all five species was not possible along these axes. However, *T. robusta* and *T. ruricola* could be separated. Also *T. robusta* and *T. hispanica* show a clear separation along PC2. Only a small overlap is found between *T. terricola* and *T. robusta*. All other species exhibit large overlaps (Fig. 3).

Tab. 2: Binary characters measured on the epigynes (I-M) and vulvae (N-P) of *T. hispanica* (n = 4), *T. robusta* (n = 6), *T. ruricola* (n = 21), *T. spinipalpis* (n = 7), and *T. terricola* (n = 10).

Binary characters			
Epigyne	I	inner margin of the helmet-shaped broadenings of the vulva	(1) straight (2) bowed/convex
	J	course of the inner margins of the helmet-shaped broadenings of the vulva	(1) margins parallel (2) margins divergent
	K	helmet-shaped broadenings of the vulva	(1) clearly visible (2) distally pointed
	L	shape and length of the dark markings anterior to the transverse pockets (= posterior part of the helmet-shaped broadenings of the vulva	(1) margins nearly parallel and extending to the apical edge of the basal part of the copulatory ducts (without appendices) (see Fig. 5) (2) margins bowed and shorter
	M	appendices of the basal part of the copulatory ducts	(1) clearly visible through the epigynal plate (2) large, nearly extending to area where the septum turns beneath the transverse pockets (3) small
Vulva	N	appendices of the spermathecae	(1) large (2) none or small
	O	lateral enlargement of the copulatory duct	(1) large/clearly visible
	P	copulatory duct	(1) short/massive with constriction (2) more or less long/thin without constriction

Hierarchical Cluster Analysis

The ordination of binary variables on the epigyne (Fig. 1 & 2, Tab. 2) showed that a reliable separation of all five *Trochosa* species is possible (Fig. 4 a). In most cases the species can be separated by the epigynal characters (Fig. 1, Tab. 2). If determination using these characters is not possible, then the characters of the vulvae (Fig. 2, Tab. 2) allow clear separation between the species. Unfortunately, the vulva data of *T. hispanica* was excluded from the analysis due to high intraspecific variation. The examination of the remaining data set showed a clear separation between the species (Fig. 4 b).

T. ruricola can be separated from all other *Trochosa* species by the convex formed inner margins of the helmet-shaped broadenings of the vulva (I), the short appendices on the spermathecae (N) and the short and massive copulatory ducts with a clearly visible constriction (P) (Fig. 5, I a & b).

T. hispanica has, in contrast to all other Central European *Trochosa* species, uniquely formed, dark markings anterior to the transverse pockets (L) and very bright helmet-shaped broadenings of the vulva (K) (Fig. 5, II a).

T. terricola has large appendices on the basal part of the copulatory ducts (M) which shine through the epigyne plate and are even clearly visible in the unprepared epigyne (Fig. 5, III a & b).

The remaining two species can be separated by the course of the inner margins of the helmet-shaped broadenings of the vulva (J) which are parallel in *T. robusta* (Fig. 5, IV a & b) and diverge forwards in *T. spinipalpis* in most cases. However, in 3% of the *T. spinipalpis* specimens the inner margins are parallel as in *T. robusta*. In comparison to *T. robusta*, *T. spinipalpis* has long appendices on the spermathecae (N) (Fig. 5, V b) while the appendices are short in *T. robusta* (Fig. 5, IV b).

Dentition

The dentition characters of the inner and outer row of the cheliceral margins overlap among the species (Tab. 3). Intraspecific differences are also apparent in the dentition. Furthermore, in all species, except *T. hispanica*, the dentition of the cheliceral margins varies even within single specimen (Tab. 3).

Tab. 3: Dentition characters of *T. hispanica* (n = 28), *T. robusta* (n = 43), *T. ruricola* (n = 48), *T. spinipalpis* (n = 34) and *T. terricola* (n = 53) in percent. Notation: left inner row – left outer row / right inner row – right outer row; inner row = posterior cheliceral tooth; outer row = anterior cheliceral tooth (ROBERTS 1995, p. 15).

species	dentition of cheliceral margins						
	3-3/3-3	2-3/2-3	2-2/2-2	3-3/2-3	2-2/2-3	1-2/2-3	1-3/2-3
Trochosa hispanica	100						
Trochosa spinipalpis	55,88	35,29		8,82			
Trochosa ruricola		79,17	4,17	10,42	4,17		
Trochosa robusta	97,67			2,33			
Trochosa terricola		90,57			3,77	1,89	3,77

Discussion

In this study we analysed 207 female *Trochosa* specimens originating from Austria, Germany, Greece, Italy, Russia and Switzerland in order to guarantee general statements about reliable separating characters within the five Central European *Trochosa* species.

Several authors have proposed a number of potential separating characters (concerning dentition, body colouration, habitat and presents of males) to which we will refer in the following discussion.

Our results about the dentition of the cheliceral margins agree with the findings of BUCHAR (1959,

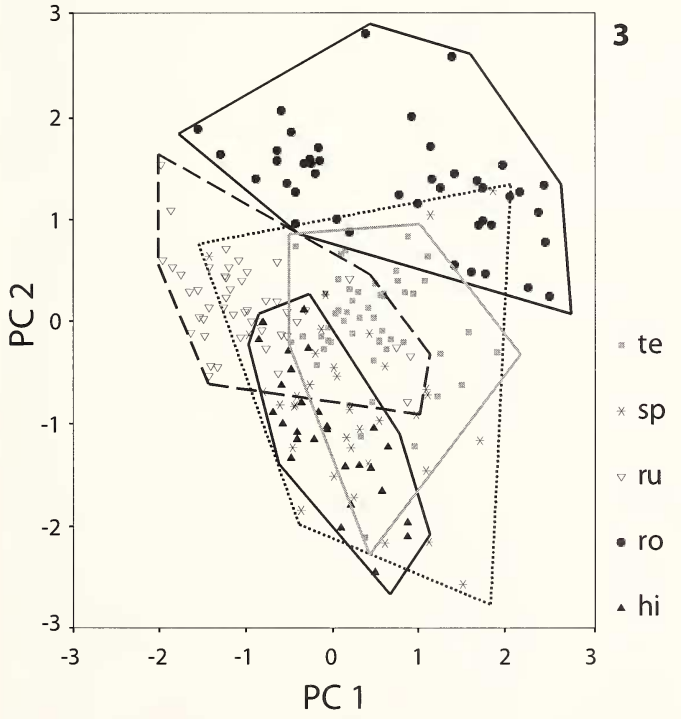

Fig. 3: Scatter plot of scores resulting from Principal Components Analysis with continuous characters representing *Trochosa* females on the two components axes (PC 1-2). te = *Trochosa terricola*; sp = *T. spinipalpis*; ru = *T. ruricola*; ro = *T. robusta*; hi = *T. hispanica*.

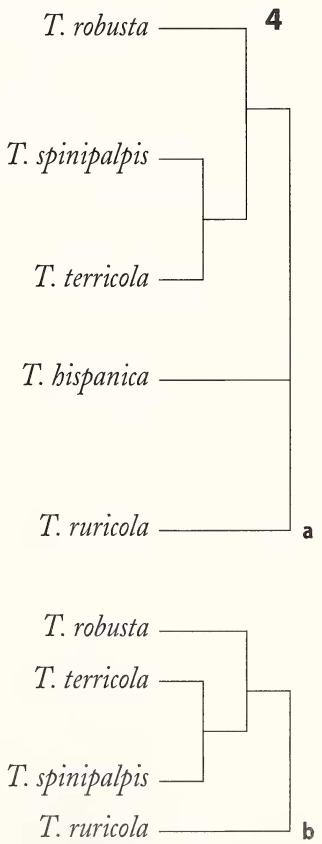

Fig. 4: Dendrogram illustrating the taxonomical separation of *Trochosa* females according to Hierarchical Cluster Analysis on **a**, epigynal and **b**, vulval characters.

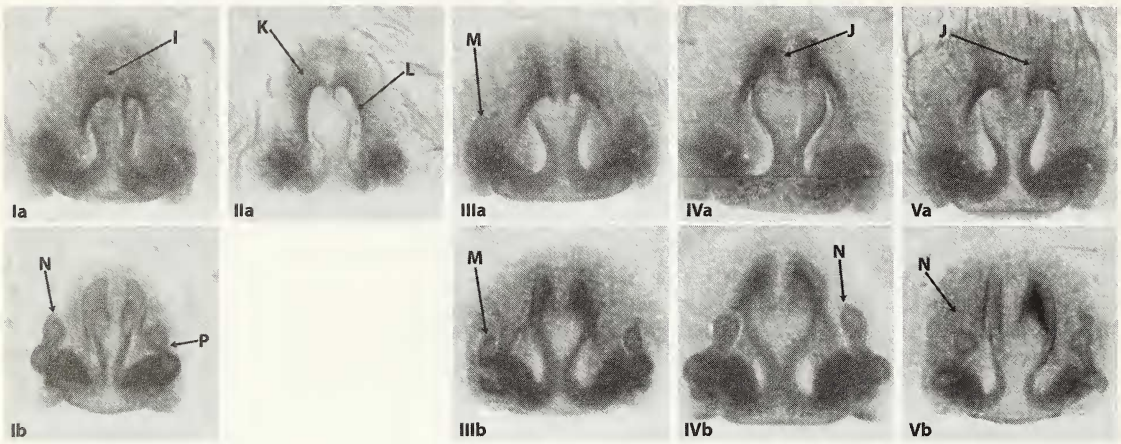

Fig. 5: Photographs of a, epigynes and b, vulvae of I, *T. ruricola*, II, *T. hispanica*, III, *T. terricola*, IV, *T. robusta* and V, *T. spinipalpis*. I = inner margin of helmet-shaped broadenings of vulva, J = course of the inner margin of the helmet-shaped broadenings of the vulva, N = appendix of the spermathecae, P copulatory duct, M = appendix of the basal part of the copulatory ducts, L = shape of the dark marks anterior to the transverse pockets and K = helmet-shaped broadenings of the vulva.

Tab. 1), ENGELHARDT (1964, Tab. 4) and MILASOWSZKY et al. (1998) that this character cannot be used for separation of *Trochosa* females due to high variability. Differences in the dentition occur not only within species/populations but also within the left and right chelicera of single specimens.

ENGELHARDT (1964) preferred body colouration as a separation criterion: "Lediglich die Färbung ist ein sicheres beiden Geschlechtern eigenes Unterscheidungsmerkmal der vier Arten". However, he also mentioned in this study that: "...die Körperfarbe, ein Kennzeichen, das bei konserviertem Material nicht mehr voll brauchbar ist." We entirely concur with Engelhardt that body colouration is useful for determining live material of *Trochosa* and that it is problematic for stored material. Colouration changes in relation to storage time and storage medium have been documented by MILASOWSZKY et al. (1999) and LOCKET & MILLIDGE (1951). Therefore, colouration could not be used as a separating character in our study, since we exclusively examined museum specimens stored in alcohol.

DAHL & DAHL (1927) favoured habitat and natural history characters:"...wir sind bei der Unterscheidung der Arten hauptsächlich auf die Unterschiede im Vorkommen und in der Lebensweise angewiesen." However, separation according to life pattern is suitable when live spiders can be observed in the field, but not for museum material. The usefulness of such traits for determination is thus greatly diminished.

A common method of determining female spiders is to assign them to co-occurring males of the same genus. This method is very problematic because of the co-occurrence of other *Trochosa* species. HÄNGGI et al. (1995) showed, for example, that *T. terricola*, the most common of the five *Trochosa* species, occurred in 82% of the sites of *T. robusta*, in 66% of the areas of *T. spinipalpis* and in 45% of the areas of *T. ruricola*. *T. ruricola* also occurs in 42% of the areas of *T. spinipalpis*. In other words, ecological preferences and the occurrence of specific males may serve as indications, but cannot ensure an accurate determination of "unknown" females.

The result of the PCA performed in this study confirms the results of MILASOWSZKY et al. (1998) that the females of *T. robusta* and *T. ruricola* can be separated by morphometrical characters. However, the separation of these two species was, in this study, not as clear as it was in MILASOWSZKY et al. (1998). We obtained a clear separation only for *T. robusta* and *T. hispanica* in the actually studied material along PC2. All other species showed large overlaps and could therefore not be separated by the morphometrical features we used in our study.

The present study, however, shows that a clear determination of the investigated *Trochosa* material is possible by examination of nominal morphological characters. These characters are taken from the epigyne and the vulva. In summary, we identified at least seven characters that allow a clear separation of the five *Trochosa* species. These characters are 1, the form of the inner margin of the helmet-shaped

broadenings of the vulva; 2, the course of the inner margins of the helmet-shaped broadenings of the vulva; 3, the size of the appendix of the spermathecae; 4, the shape of the copulatory duct; 5, the occurrence and size of the appendix of the basal part of the copulatory ducts; 6, the shape of the dark marks anterior to the transverse pockets; and 7, the appearance of the helmet-shaped broadenings of the vulva. Nevertheless, in most cases a combination of epigynal and vulval characters is the best way to guarantee a clear identification of females of the five Central European *Trochosa* species.

Identification key

1 Helmet-shaped broadenings clearly visible and their inner margins convex (Fig. 5, I a); copulatory ducts short, massive with a constriction (Fig. 5, I b) .... ***ruricola***

– Helmet-shaped broadenings nearly invisible (Fig. 5, II a) or the inner margins of the helmet-shaped broadenings straight (Fig. 5, f. e. V a) *2*

2 Helmet-shaped broadenings nearly invisible; dark marks anterior to the transverse pockets long and nearly parallel (Fig. 5, II a)..... ***hispanica***

– Not like this *3*

3 Appendices of the basal part of the copulatory duct large and clearly seen through the epigyne (Fig. 5, III a & b)..... ***terricola***

– Not like this *4*

4 inner margins of the helmet-shaped broadenings of the vulva parallel; copulatory ducts with only small appendices (Fig. 5, IV a & b) ..... ***robusta***

– inner margins of the helmet-shaped broadenings of the vulva (in most cases) divergent; copulatory ducts with large appendices (Fig. 5, V a & b)... ***spinipalpis***

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